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With or without trees: Resistance and resilience of soil microbial communities to drought and heat stress in a Mediterranean agroforestry system

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ABSTRACT

Soil microbial communities in Mediterranean agroecosystems experience long drought periods that are typically combined with heat and frequently interspersed with rapid rewetting events. Agroforestry systems are of growing interest and viewed as possible alternative to conventional cropping systems in the context of climate change. Our aim was to evaluate the resistance and resilience of soil microbial communities against drought with or without heat stress at different distances from the tree row in an agroforestry system as compared to a conventional cropping system. Soils were sampled at several distances from the tree row in a 21-year-old walnut agroforestry system and a contiguous conventional crop in Southern France. We simulated two cycles of drying-rewetting under controlled conditions and applied three distinct treatments: control (without stress), drought and drought combined with heat stress. We monitored microbial respiration over the incubation period. The inorganic N and microbial biomass C, N and P contents (MBC, MBN and MBP) were assessed during the drying period (resistance), just after rewetting and at the end of the experiment (resilience), while bacterial and fungal abundances were measured at the end of the resistance period. We demonstrated that an agroforestry system can induce spatial heterogeneity in soil microbial biomass and functions under control conditions. Microbial biomass and activity, soil organic matter (SOM) and mineral N levels increased on the tree row. This spatial heterogeneity pattern was preserved for soil microbial response to drought combined or not with heat. Microorganisms sampled in the middle of the interrow or in the conventional crop exhibited highest biomass resistance and lowest resilience when facing combined drought and heat stress. Soil microbial biomass resistance and resilience were similar whatever the spatial position when microorganisms had to deal with drought stress only. Our findings suggested that despite higher SOM content, microbial biomass and activity at and near the tree row, the legacy effect of the tree row did not lead to higher ecological stability under stressful climatic conditions. We also demonstrated that soil microorganisms can considerably change their stoichiometry depending on the stress treatment. Soil microorganisms showed elevated MBC:MBN, MBC:MBP and variable MBN:MBP during the resistance period. A high stoichiometric flexibility of microorganisms was observed when exposed to drought stress only, while stoichiometric changes were irreversible when exposed to combined drought and heat stress.

1. Introduction

Climate change is expected to increase the frequency of drought and extreme events, with either high rainfall or elevated temperatures (Stocker et al., 2013). This is especially true in the Mediterranean region. Under Mediterranean conditions, soil microorganisms must survive long drought periods, which are typically combined with heat and frequently interspersed by rapid rewetting events related to storms in summer, autumn or spring (Gibelin and Déqué, 2003). Drying-rewetting events can affect the structure of soil microbial communities and modify ecosystem C and nutrient fluxes with, for instance, an increased mineralization of soil organic matter (SOM), especially in tilled soils (Banning and Murphy, 2008; Denef et al., 2001; Fierer et al., 2003; Fierer and Schimel, 2002; Mikha et al., 2005). Temperature also affects soil microbial activity and their stoichiometry (Xu and Yuan, 2017). Soil microbial stoichiometry could help to understand the functioning

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of microbial communities and their demand in energy (C) and nutrients (N and P) (Cleveland and Liptzin, 2007). Change in elemental ratios of soil microbial biomass could reflect resource availability, the use efficiency of C, N and P by the microbial biomass and ultimately alter C, N and P cycling (Mooshammer et al., 2014). Recently, the meta-analysis of Xu and Yuan (2017) highlighted that the effect of warming on soil MBC and MBN and their ratio (MBC:MBN) was dependent on the magnitude and duration of heat. An intermediate increase in temperature (1–2 °C) tended to increase MBC and, to a greater extent, MBN, leading to a lower MBC:MBN ratio. However, the effects of drought stress with or without heat on soil microbial stoichiometry have rarely been studied.

The responses of soil microorganisms to stress conditions can be evaluated with two components of ecological stability: resistance (RS, the ability of a community property or process to remain unchanged in the face of a specific disturbance) and resilience (RL, the ability of a community property or process to recover after a specific disturbance) (Allison and Martiny, 2008; de Vries and Shade, 2013; Pimm, 1984). Several indices have been developed to evaluate the RS and RL of microorganisms in response to stress (Banning and Murphy, 2008; Orwin and Wardle, 2004). Both the RS and RL of soil microorganisms can be affected by nutrient availability and/or the SOM level, which vary with agricultural practices (Kaisermann et al., 2013). Fungal-based food webs, such as those occurring in grassland or forest ecosystems, tend to be more resistant to drought than bacterial-based food webs, such as those found in croplands (de Vries et al., 2012). Alley-cropping agroforestry systems are composite ecosystems composed of trees and herbaceous strips between the tree rows that are comparable to grasslands and are thus expected to be dominated by fungal energy channels; in contrast, the cropped interrows are expected to be dominated by bacterial energy channels. Thus, differences in soil microbial functioning may occur across a spatial gradient with distance from the tree row. Complex agroecosystems are acknowledged as more adaptable than conventional cropping (CC) systems to climate change and particularly temperate agroforestry systems that are of growing interest because of their potential capacities to store C in plant and soil compartments (Abbas et al., 2017; Altieri and Nicholls, 2017; Cardinael et al., 2015; Jose, 2009; Schoeneberger et al., 2012; Verchot et al., 2007). However, few studies have focused on the soil microbial response to climate change in agroforestry systems, and none has addressed their microbial biomass stoichiometry. Rivest et al. (2013) observed, a higher RL of MBC, 5 m from a windbreak than in an adjacent CC and a lower RL 5 m from a tree row in a tree-based intercropping site than in an adjacent CC. The authors attributed these different resilience responses to the differences is soil texture between the two sites, suggesting that heavier textured soils may reflect higher microbial diversity. To our knowledge, no previously published work has considered the spatial heterogeneity of soil functioning within an agroforestry plot, which may occur in these systems. Indeed, distance from the tree may modify the soil microclimate, OM input and thus impact soil microbial RS and/or RL against drought and/or heat stress.

In this study, we hypothesized that i) a spatial gradient of soil microbial biomass and mineralization function occurs between the tree row and the middle of the cultivated interrow under optimal and stressed conditions, ii) compared to a CC system, an agroforestry system can improve the ecological stability (RS and RL) of the soil microbial communities under drought and/or heat stress and iii) changes in soil microbial biomass due to stresses are related to their stoichiometry. To test these hypotheses, a sampling design was established in a 21-year-old Mediterranean agroforestry site. This design considered a spatial gradient from the tree row to the middle of the cultivated interrow and was compared with an adjacent CC system managed with the same agricultural practices. The effects of two cycles of drought stress or combined heat and drought stress on soil microbial biomass respiration and stoichiometry were determined in incubation. RS and RL indices were calculated for the C, N and P contents of the microbial biomass and for the C mineralization rates after one month of stress and at the end of the incubation period.

2. Materials and methods

2.1. Site description

The experimental site of Restinclières is located at Prades-le-Lez, 15 km north of Montpellier, Southern France (43°42′15 N, 3°51′41 E). It has a subhumid Mediterranean climate and an average temperature of 15.4 °C and average annual rainfall of 658 mm over the years 2000–2016 (Fig. S1). The rainfall is exhibiting considerable variability, ranging from 419 mm in 2007 to 1127 mm in 2003, and is unevenly distributed within the year, with rather long periods of drought in Summer and heavy storm events in Autumn and sometimes in August-early September. Over the years 2014–2016, each year has been exhibiting 1–2 drought periods (without significant rainfall) of 23–44 days between May and August, with maximum daily air temperatures up to 33.6–36.5 °C (according to Montpellier weather station records). The soil is a deep Fluvisol (WRB, 2007) with a silty-clay texture. In February 1995, hybrid walnut trees (*Juglans regia* × *nigra* cv. NG23) were planted 4 m apart along east-west rows, with an interrow distance of 13 m. The initial planting density was 220 trees ha^{-1}, but the plot was thinned in 2004 down to the present density of 110 trees ha^{-1}. The tree rows (2-m wide) are covered with spontaneous herbaceous vegetation, as they have not been plowed or treated with herbicides since 1995. Each interrow is plowed to 20-cm depth every year before the winter crop is sown and is fertilized at an approximate rate of 150 kg N ha^{-1} yr^{-1} (as ammonitrate). Most of the time since 2000 durum wheat was cultivated, while pea was introduced since 2010 every 3 years (2010, 2013, 2016), and barley since 2015. Over the 2014–2016 period, a durum wheat (*Triticum turgidum durum* cv. Claudio)-barley (*Hordeum vulgare* cv. Augusta)-pea (*Pisum sativum* cv. Igloo) rotation was practiced on this 4.6-ha agroforestry plot and on the adjacent 1.4-ha CC plot, which has been managed exactly in the same way as the cultivated interrows since the experimental site was established. All crops are sown between the end of October and the beginning of December and harvested by early Summer.

2.2. Soil sampling

The soil sampling was conducted in April 2016, when pea was grown in the CC plot and in the interrow of the agroforestry plot. For the CC plot, five sampling areas, each 1 m^2, were defined at 10-m intervals along two transects in the central part of the plot, yielding five pseudo replicates. One composite soil sample (based on 4 soil cores, 8 cm in diameter) was collected at each area from the top 15 cm. For the agroforestry plot, 20 soil samples were collected following the same procedure. Five transects, each considered as a pseudo replicate, were sampled from the tree row to the middle of the interrow to define a spatial gradient. This spatial gradient (1 m wide and 7.5 m long in total) consisted of 4 contiguous sampling areas perpendicular to the tree row on the north side of the row (Fig. S2). The first area (A) was the only one located at the tree row (2 m^2, with its center located on the axis of the tree row 1 m from the nearest tree). The other three sampling areas were located in the cultivated interrow; the second area (B) was located between 1 and 2 m from the middle of the tree row, the third area (C) between 2 and 4 m from the middle of the tree row and the last area (D) between 4 and 6.5 m from the middle of the tree row. Fresh soils were passed through a 2 mm sieve and stored at 4 °C in the dark during one month prior to the beginning of the incubation. Soil physical and chemical characteristics measured on all the composite samples collected on the various sampling areas are summarized in Table 1.

Table 1
Physical and chemical characteristics of the soil used in the incubation experiment. Texture data presented here were determined after soil decarbonation. Data represent mean values (n = 5) for each position: tree row (A), between 1 and 2 m from the middle of the tree row (B), between 2 and 4 m from the middle of the tree row (C), between 4 and 6.5 m from the middle of the tree row (D) and in the CC system. Within each line, different letters indicate significantly different values between positions ($P < 0.05$).

	A	B	C	D	CC
Silt (g kg^{-1})	157 ^{a}	165 ^{ab}	157 ^{a}	158 ^{a}	208 ^{b}
Sand (g kg^{-1})	109 ^{a}	101 ^{a}	105 ^{a}	103 ^{a}	76 ^{a}
Clay (g kg^{-1})	178 ^{a}	173 ^{a}	186 ^{a}	184 ^{a}	177 ^{a}
$CaCO_3$ (g kg^{-1})	539 ^{a}	545 ^{a}	540 ^{a}	539 ^{a}	527 ^{a}
pH (water)	8.4 ^{a}	8.5 ^{b}	8.6 ^{b}	8.6 ^{b}	8.6 ^{b}
CEC ($cmol_+$ kg^{-1})	14.7 ^{a}	13.0 ^{ab}	12.5 ^{ab}	12.4 ^{ab}	11.8 ^{b}
C_{org} (mg kg^{-1})	20.5 ^{b}	15.5 ^{ab}	15.1 ^{ab}	13.9 ^{a}	15.2 ^{ab}
N_{tot} (mg kg^{-1})	1.51 ^{a}	1.04 ^{b}	0.95 ^{b}	0.91 ^{b}	0.94 ^{b}
P_{Olsen} (mg kg^{-1})	8.3 ^{b}	5.8 ^{a}	5.2 ^{a}	5.6 ^{a}	5.8 ^{a}

2.3. Soil incubation and stress assays

A soil incubation experiment was conducted in the growth chambers of the Microcosms platform of the CNRS Montpellier Ecotron (www.ecotron.cnrs.fr). The microcosms were made of 250-cm^3 glass bottles filled with 100 g of equivalent dry soil at 60% water holding capacity (WHC) and sealed with a rubber septum plug. Soils for all treatments were first maintained for 3 weeks at 25 °C in the dark at 60% WHC in order to stabilize the soil microbial activities prior to the start of the experiment. This preincubation was performed in presence of an alkali trap (1 M NaOH), and soil moisture was readjusted to 60% WHC by adding deionized water if necessary. A total of 225 microcosms were prepared, composed of 5 positions (A, B, C, D, CC), each with 5 replicates and 3 treatments at 3 sampling dates (day 28, 32 and 105) (Fig. 1). The three treatments were: the control treatment at 25 °C (C25), the drought stress treatment at 25 °C (S25), and the combined drought and heat stress treatment at 40 °C (S40). Microcosms were distributed in 2 ventilated culture cabinets set at 25 ± 0.3 °C and 40 ± 0.3 °C. Control soils (C25) were maintained at 25 °C and at 60% of their WHC during all the experiment. Two cycles of drying-rewetting events were imposed, the first drought period lasting for 28 days, while the second lasted for 24 days (Fig. 1). These durations were in accordance to the length of drought periods commonly experienced at this site, as mentioned above. Soils from the S25 treatment were kept at 25 °C during the entire incubation, while the S40 treatment soils were kept at 40 °C for 76 days and were then transferred back to 25 °C after the last rewetting event on day 77. For the drying periods, the bottles were kept open for 8 days to air dry the soil at either 25 or 40 °C. A low soil humidity level (< 2.5% w:w) was maintained during the whole drought stress period by placing 15 g of silica gel in a plastic bottle in the microcosm (Kaisermann et al., 2013). The silica gel was changed weekly. All microcosms were weighted weekly to monitor their soil water content. The moisture content was adjusted to the control level at the end of each of the two drought stress periods (days 29 and 77), while control samples were maintained at 60% WHC during the whole experiment. The RS was measured based on the microbial biomass and respiration on day 28 just before the first rewetting event, while the RL was assessed on day 105, i.e., 28 days after the last rewetting event (Fig. 1). Another sampling was performed on day 32 in order to capture the microbial activity immediately after rewetting. At each of these three sampling dates (28, 32 and 105 days), 75 microcosms were destructively sampled: 25 samples (5 replicates per position A, B, C and D, CC) for each treatment (C25, S25 and S40). Moist soil samples were stored at 4 °C until the microbial biomass analysis and mineral N analysis were performed.

2.4. Soil microbial and chemical analyzes

Microbial respiration was measured using 1 M NaOH traps placed in each microcosm. Traps were collected 14, 21, 28, 32, 36, 43, 50, 53, 60 (only for C25, because of the air-drying period for treatments S25 and S40), 67, 74, 77, 80, 84, 91, 98 and 105 days after the beginning of the experiment. The concentrations of trapped CO_2 were measured with a potentiometric titration system (Metrohm, 848 Titrino Plus). Microbial biomass C (MBC), N (MBN) and P (MBP) were quantified on day 28, 32 and 105 using the chloroform fumigation-extraction technique (Vance et al., 1987). Briefly, 10 g (for MBC and MBN) or 2 g (for MBP) of equivalent dry soil was exposed to chloroform vapor for 24 h and then extracted with 40 cm^3 of 0.025 M K_2SO_4 (for MBC and MBN) or with 40 cm^3 0.5 M $NaHCO_3$ (for MBP), shaken for 45 min, centrifuged (10 min at 2683 *g*) and then filtered through 0.22-µm PTFE filters. Organic C and N concentrations in the extracts were measured by a TOC/TON analyzer (OI-Analytical, Aurora 1030, College Station, USA). Inorganic P concentrations in the extracts were quantified colorimetrically using the malachite green method (Ohno and Zibilske, 1991). Microbial biomass C, N, and P (MBC, MBN and MBP) contents were calculated from the difference between the chloroform-fumigated and non-fumigated samples. We applied a conversion factor of 0.45 for MBC and MBN and a conversion factor of 0.4 for MBP (Jenkinson, 2004). Total C of non-fumigated samples was used to represent dissolved organic carbon (DOC). The soil mineral N was extracted from 0.5 g equivalent dry soil with 5 cm^3 of 1 M KCl solution. The NO_3^- and NH_4^+ were determined by continuous flow colorimetry (SKALAR 3000 auto analyzer). Additional soil analyzes were performed by the national routine soil testing lab of INRA at Arras (LAS, France) for all the soils prior to the incubation experiment: soil texture was determined after

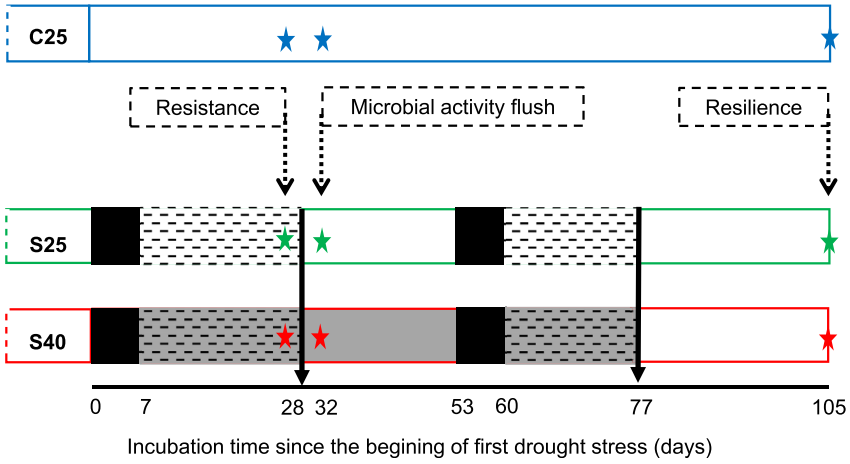

Fig. 1. Schematic representation of the incubation design, showing the control treatment (C25, in blue), the drought stress treatment at 25 °C (S25, in green) and the combined drought and heat stress treatment at 40 °C (S40, in red). The white background indicates an incubation temperature of 25 °C, and the dark gray background indicates an incubation temperature of 40 °C. Black squares represent the microcosm air-drying periods, and dotted areas represent the drought periods. Solids arrows represent microcosm rewetting days. Stars and dotted arrows represent the 3 sampling dates. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

$CaCO_3$ dissolution (following the NF X 31–107 standard protocol) and pH was determined in water (NF ISO 10390). The cation exchange capacity (CEC) was determined using the cobaltihexamine chloride method and analyzed through inductively coupled plasma-atomic emission spectrometry (ICP-AES) (NF X 31–130). Soil organic C (SOC) and total N (N_{tot}) were determined by dry combustion (NF ISO 10694 and NF ISO 13878). Available P content was extracted using the Olsen method and analyzed colorimetrically (NF ISO 11263). All measured variables were expressed relative to the dry soil mass.

2.5. Extraction of total soil DNA for bacterial and fungal abundance determinations

Deoxyribonucleic acid (DNA) was extracted from soil subsamples of 250 mg ± 1 mg according to the method described by Tournier et al. (2015). A washing step with 5.5 M guanidine thiocyanate was added to the protocol in order to remove humic acids from the soils, as humic acids can be quantitative polymerase chain reaction (qPCR) inhibitors. DNA quantification was then estimated using PicoGreen fluorescence. The bacterial 16S and fungal 18S genes were selected as phylogenetic markers and were assessed by qPCR. The Purification RP-Cartridge–Gold 341F and 515R primers were used to amplify the bacterial 16S gene (López-Gutiérrez et al., 2004), and the Purification RP-Cartridge–Gold FR1 and FR390 primers were used to amplify the fungal 18S gene (Vainio and Hantula, 2000). The 10 mm^3 qPCR mix comprised 2 mm^3 of soil microbial DNA at 1.25 ng mm^{-3}, 5 mm^3 of Sso Advanced BioRad master mix, 2 mm^3 of molecular grade H_2O and 0.5 mm^3 of each primer. A standard curve with known concentrations of *Pseudomonas aeruginosa* genomic DNA and *Hebeloma cylindrosporum* H7 DNA were used for 16S and 18S quantification, respectively. First, an initial DNA denaturation and high-efficiency enzyme activation of 2 min at 98 °C was performed. Then, 39 cycles of PCR were performed with the CFX96 Real time System Thermocycler (BioRad, Hercules, CA, USA) as follows: 5 s at 98 °C, 30 s at 60 °C for 16S and 58 °C for 18S and 30 s at 72 °C.

2.6. Soil microbial resistance and resilience indices

To evaluate the functional RS and RL of soil microbial communities exposed to drought and heat stress, the following indices were used according to Orwin and Wardle (2004):

$$RS = 1 - [(2 \times |D28|)/(C28 + |D28|)]$$

where $D28$ is the difference between the soil C mineralization rates (expressed in mg C-CO_2 kg^{-1} soil day^{-1}) or the microbial biomass C, N, or P content (expressed in mg C, N or P kg^{-1} soil), in the control soil ($C28$) and in the stressed soil (drought or combined drought and heat stress) on day 28, i.e. at the end of the first drought period of the incubation experiment.

The RL index for the soil C mineralization rates or microbial biomass C, N, or P was calculated at the end of the incubation period (i.e., on day 105), according to:

$$RL = [(2 \times |D28|)/(|D28| + |D105|)] - 1$$

where $D105$ is the difference between the soil C mineralization rates (expressed in mg C-CO_2 kg^{-1} soil day^{-1}) or microbial biomass C, N, or P content (expressed in mg C, N or P kg^{-1} soil) in the control soil and in the stressed soil (drought or combined drought and heat stress) on day 105. The RS and RL indices range between −1 and +1. A value of +1 indicates maximum RS (no impact of disturbance) or RL (complete recovery after disturbance), and lower values indicate less RS or RL. Negative values of the RS index indicate a change greater than 100% in the response variable compared to that in the control soil. If the absolute value of $D105$ is higher than the absolute value of $D28$, the RL index will be negative.

2.7. Metabolic quotient

The metabolic quotient (qCO_2), defined as the C respired per unit of MBC (Anderson and Domsch, 1993), was calculated for each sampling date, according to the following equation:

$$qCO_2 = CO_2/MBC$$

where CO_2 is the respiration rate expressed as mg C-CO_2 kg^{-1} soil day^{-1}, and MBC is the microbial biomass C expressed as mg C kg^{-1} soil.

2.8. Statistical analyzes

All statistical analyzes were performed with R software v.3.2.3 (R Development Core Team, 2016). The effects of position and treatment on the microbial biomass C, N, and P contents, C mineralization rates, N mineral contents, DOC contents, RS and RL indices, metabolic quotient, 16S and 18S abundances and means stoichiometric ratios for each sampling date were tested by two-way ANOVA (position × treatment). One-way ANOVA were performed to analyze, within a treatment, the effect of each position and vice versa. If significant, a Tukey post hoc test was used for pairwise multiple comparisons ($P < 0.05$). Comparison to the control for N mineral contents, DOC contents, 16S and 18S abundances were tested by Student-test. Normal distribution of residuals and homogeneity of variance were tested by the Shapiro and Bartlett tests. If necessary, data were Box-Cox transformed (MASS package). A redundancy analysis (RDA) was performed to assess the impact of decrease in 16S and 18S copy number, DOC, MBC, MBN, MBP and microbial respiration on microbial RS. Linear models were performed on MBC *vs* MBN, MBC *vs* MBP and MBN *vs* MBP. If regressions were significant, ANCOVA analyzes followed by Tukey post hoc tests were performed in order to determine differences between the slopes of the microbial biomass C:N (MBC:MBN), C:P (MBC:MBP) and N:P (MBN:MBP) ratios according to the treatment. Linear models were also performed on microorganisms' stoichiometric ratios *vs* resource ratios. Data were analyzed using principal component analysis (PCA) with the FactoMineR package. Significant differences between the levels within each factor based on PCA were determined by using the Monte Carlo permutation test based on Bray-Curtis distance (Biodiversity R package).

3. Results

In control soils (C25 treatment), the tree row position (A) always exhibited significantly highest microbial activity (CO_2 mineralization, Fig. 2a, Tables 2 and 3) and biomass C, N and P contents (MBC, MBN and MBP, Table 4), DOC content (Fig. 3), mineral N concentration (Fig. 4), 16S and 18S copy numbers (Table 5). Values of all these variables at A position were most of the time approximately 1.5-fold higher than those at B position and nearly 2-fold higher than those measured at C, D and CC positions, which showed similar values. Details of microbial community responses to S25 and S40 stress treatments are presented in the next paragraphs.

3.1. Overall position and treatment effects

The PCA performed on the measured parameters before (SOC, P_{org} and N_{tot}) and during the incubation (MBC, MBN, MBP, CO_2 rates, DOC and N_{min} contents) revealed a dispersion of individuals along two axes (Fig. 5a and b). The first axis explained 43% of the total variance of the data set. There was a strong contribution ($R > 0.60$) of all forms of N, soil microbial biomass C, N and P and SOC to the first axis (Fig. 5a). The coordinates of the centroids on the first axis, as calculated for each position, showed that the tree row (A position) had positive values, while all other positions exhibited negative values (Fig. 5b). The Monte Carlo test revealed significant distinctions among the different

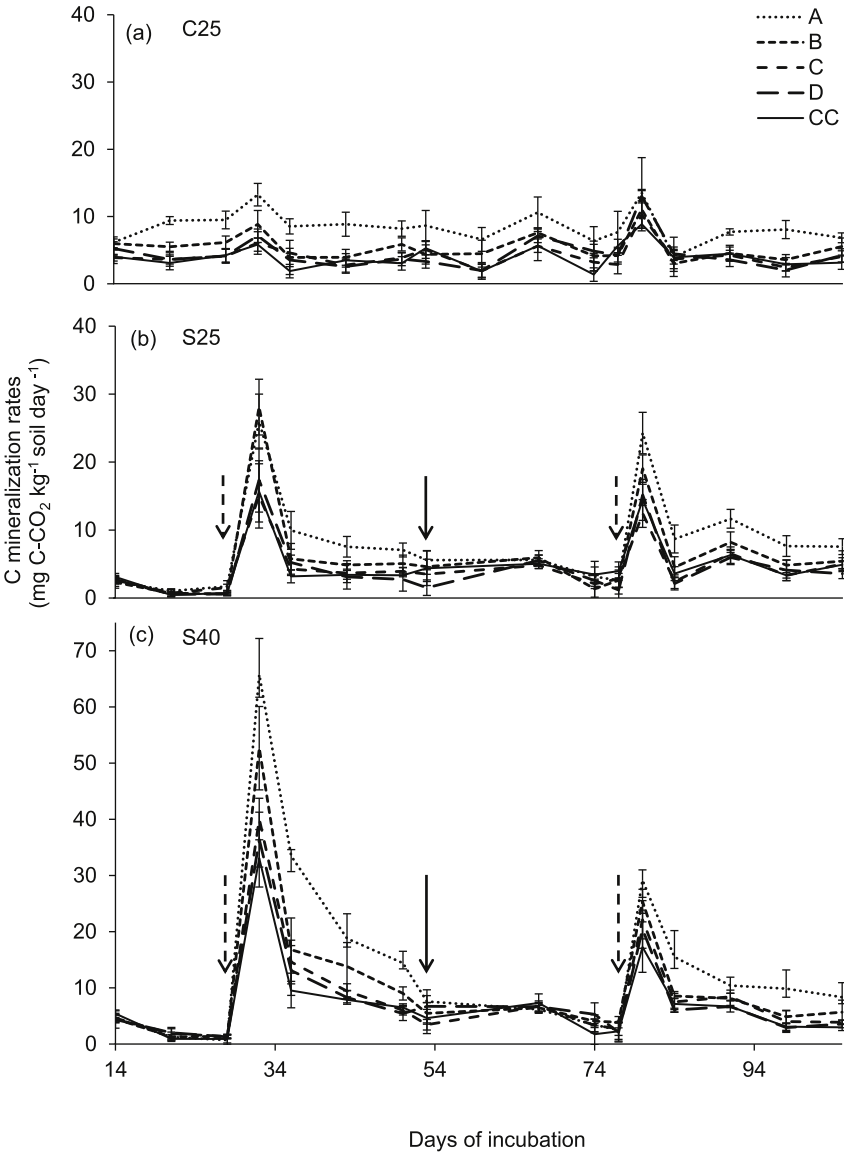

Fig. 2. Organic C mineralization rates in mg C-CO_2 kg^{-1} soil day^{-1} for the different positions (A, B, C, D and CC) for the control (C25) (a), drought stress (S25) (b) and combined drought and heat stress (S40) (c) treatments. Day 14 is the first sampling date after one week of respiration under drought conditions. Data are means ± standard deviation (n = 5). Solid arrows indicate microcosms opening days, and dotted arrows indicate rewetting days.

Table 2
Rates of C mineralization (mg C-CO_2 kg^{-1} soil day^{-1}) for the different spatial positions (A, B, C, D and CC) under control (C25), drought stress (S25), and combined drought and heat stress (S40) treatments. Resistance (RS, day 28) and resilience (RL, day 105) indices based on the organic C mineralization rates for S25 and S40 treatments are presented. Sum of squares in percentages (%) of the two-ways ANOVA for position, treatment and interaction of both (P × T) are presented at the top of the table, with levels of statistical significance: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Data represent mean values (n = 5). Within a column, different lowercase letters indicate significantly different values between positions for each treatment ($P < 0.05$). Different capital letters indicate significant differences between treatments for each variable at one date ($P < 0.05$).

	Day 28					**Day 105**				
Factor	CO_2 Rates			RS		CO_2 Rates			RL	
Position	7 ***			38 ***		58 ***			10	
Treatment	76 ***			2		3 *			6 *	
P × T	10 ***			18 **		11 *			27 **	
Position	C25 ^{A}	S25 ^{B}	S40 ^{B}	S25 ^{A}	S40 ^{A}	C25 ^{A}	S25 ^{A}	S40 ^{A}	S25 ^{A}	S40 ^{A}
A	9.50 ^{c}	1.69 ^{c}	0.71 ^{a}	0.10 ^{a}	0.04 ^{a}	6.84 ^{c}	7.56 ^{b}	8.35 ^{c}	0.80 ^{b}	0.72 ^{a}
B	6.15 ^{b}	0.59 ^{a}	1.35 ^{a}	0.05 ^{a}	0.12 ^{ab}	5.53 ^{bc}	5.41 ^{a}	5.67 ^{bc}	0.82 ^{b}	0.64 ^{a}
C	4.11 ^{a}	1.53 ^{bc}	1.36 ^{a}	0.27 ^{b}	0.22 ^{b}	4.05 ^{ab}	5.00 ^{a}	3.88 ^{ab}	0.29 ^{a}	0.75 ^{a}
D	4.14 ^{a}	0.58 ^{a}	1.28 ^{a}	0.08 ^{a}	0.19 ^{b}	4.22 ^{ab}	3.58 ^{a}	3.57 ^{ab}	0.64 ^{ab}	0.60 ^{a}
CC	4.24 ^{ab}	0.76 ^{ab}	0.91 ^{a}	0.10 ^{a}	0.12 ^{ab}	3.14 ^{a}	4.89 ^{a}	2.97 ^{a}	0.31 ^{a}	0.86 ^{a}

Table 3
Cumulative respiration (mg C-CO_2 kg^{-1} soil) and cumulative respiration normalized by SOC (mg C-CO_2 g^{-1} C) at the end of the incubation period (day 105) for the different spatial positions (A, B, C, D and CC) under control (C25), drought stress (S25), and combined drought and heat stress (S40) treatments. Sum of squares in percentages (%) of the two-ways ANOVA for position, treatment and interaction of both (P × T) are presented at the top of the table, with levels of statistical significance: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Data represent mean values (n = 5). Within a column, different lowercase letters indicate significantly different values between positions for each treatment ($P < 0.05$). Different capital letters indicate significant differences between treatments for each variable ($P < 0.05$).

	Day 105					
Factor	Cumulative respiration			Cumulative respiration normalized by SOC		
Position	53 % ***			16 % ***		
Treatment	30 % ***			44 % ***		
P × T	2 %			2 %		
Position	C25 ^{A}	S25 ^{A}	S40 ^{B}	C25 ^{A}	S25 ^{A}	S40 ^{B}
A	728 ^{b}	583 ^{b}	876 ^{c}	36.3 ^{b}	29.3 ^{a}	45.3 ^{a}
B	459 ^{a}	410 ^{a}	672 ^{b}	29.8 ^{ab}	26.7 ^{a}	43.9 ^{a}
C	343 ^{a}	320 ^{a}	557 ^{ab}	23.5 ^{a}	21.6 ^{a}	38.1 ^{a}
D	368 ^{a}	334 ^{a}	526 ^{a}	27.9 ^{ab}	25.8 ^{a}	40.0 ^{a}
CC	328 ^{a}	332 ^{a}	469 ^{a}	22.2 ^{a}	22.4 ^{a}	32.4 ^{a}

positions, as follows: A ≠ B ≠ C = D = CC. The second axis explained 19% of the total variance and was strongly, positively correlated to the DOC content and MBC:MBN ratio. The coordinates of the centroids on the second axis, as calculated for each treatment, showed that S40 had positive values, while S25 and C25 exhibited rather negative values (Fig. 5a). The Monte Carlo test revealed significant distinctions among the different treatments, as follows: C25 = S25 ≠ S40.

3.2. Organic carbon and nitrogen mineralization

At day 28, the treatment factor was the main driver of CO_2 rates (76% of the total variance explained), while at day 105, one month after the last rewetting, the position explained most of the variance (58%) (Table 2). After 28 days of drought at 25 °C (S25) or 40 °C (S40), the mineralization rates decreased by 63–92% for agroforestry positions and by 80% for the CC position (Fig. 2b and c), compared to the rates of their respective control soils (Fig. 2a). Rewetting on day 29 induced high pulses of mineralization at 25 °C which were significantly higher ($P < 0.05$, data not shown) in A and B positions than pulses observed in C, D or CC positions, which were similar. Under the S40 treatment, the respiration pulses were significantly higher than under S25 and the different positions ranked as follows: A > B > C = D = CC. At the end of the experiment, C25, S25 and S40 yielded similar mineralization rates (Table 2). The total amount of mineralized C was not significantly different between soils from C25 and S25, but it was significantly increased in S40 (Table 3). The highest total amount of C mineralized was systematically found for the tree row (A position). When accounting for soil organic C concentrations (values normalized by SOC), the

Table 4
Microbial biomass C (MBC), N (MBN) and P (MBP) contents in mg C, N or P kg^{-1} soil at different spatial positions (A, B, C, D and CC) under control (C25), drought stress (S25), and combined drought and heat stress (S40) treatments on the 3 sampling dates. Sum of squares in percentages (%) of the two-ways ANOVA for position, treatment and interaction of both (P × T) are presented at the top of the table, with levels of statistical significance: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Data represent mean values (n = 5). Within a column, different letters indicate significantly different values between positions for each variable ($P < 0.05$).

		Day 28			Day 32			Day 105		
Factor										
MBC	Position	51 ***			50 ***			54 ***		
	Treatment	28 ***			25 ***			22 ***		
	P × T	11 ***			13 ***			7 **		
MBN	Position	17 ***			27 ***			23 ***		
	Treatment	59 ***			47 ***			44 ***		
	P × T	8 ***			15 ***			15 ***		
MBP	Position	1 ***			15 **			20 ***		
	Treatment	27 ***			24 ***			19 ***		
	P × T	8			17 **			14 *		
	Position	C25 ^{A}	S25 ^{A}	S40 ^{B}	C25 ^{A}	S25 ^{A}	S40 ^{B}	C25 ^{A}	S25 ^{A}	S40 ^{B}
MBC	A	367 ^{b}	303 ^{c}	192 ^{b}	413 ^{b}	382 ^{c}	235 ^{b}	314 ^{c}	323 ^{c}	224 ^{b}
	B	211 ^{a}	191 ^{b}	115 ^{a}	224 ^{a}	246 ^{b}	159 ^{a}	215 ^{b}	210 ^{b}	150 ^{a}
	C	180 ^{a}	147 ^{a}	100 ^{a}	202 ^{a}	186 ^{a}	157 ^{a}	168 ^{a}	187 ^{ab}	132 ^{a}
	D	170 ^{a}	136 ^{a}	115 ^{a}	196 ^{a}	176 ^{a}	146 ^{a}	176 ^{a}	151 ^{a}	128 ^{a}
	CC	166 ^{a}	151 ^{a}	126 ^{a}	190 ^{a}	164 ^{a}	148 ^{a}	174 ^{a}	185 ^{ab}	124 ^{a}
MBN		C25 ^{A}	S25 ^{B}	S40 ^{C}	C25 ^{A}	S25 ^{A}	S40 ^{B}	C25 ^{A}	S25 ^{A}	S40 ^{B}
	A	60 ^{c}	23 ^{c}	6 ^{b}	58 ^{b}	60 ^{b}	19 ^{b}	47 ^{b}	51 ^{b}	19 ^{a}
	B	30 ^{b}	12 ^{b}	5 ^{b}	33 ^{a}	32 ^{a}	10 ^{a}	32 ^{a}	30 ^{a}	15 ^{a}
	C	27 ^{ab}	11 ^{ab}	3 ^{a}	30 ^{as}	28 ^{a}	11 ^{a}	29 ^{a}	28 ^{a}	14 ^{a}
	D	27 ^{ab}	9 ^{ab}	2 ^{a}	28 ^{a}	27 ^{a}	13 ^{ab}	28 ^{a}	23 ^{a}	15 ^{a}
	CC	20 ^{a}	6 ^{a}	3 ^{ab}	27 ^{a}	25 ^{a}	15 ^{ab}	24 ^{a}	24 ^{a}	14 ^{a}
MBP		C25 ^{A}	S25 ^{B}	S40 ^{B}	C25 ^{A}	S25 ^{B}	S40 ^{C}	C25 ^{A}	S25 ^{A}	S40 ^{B}
	A	18.5 ^{b}	6.8 ^{b}	5.4 ^{b}	16.1 ^{b}	13.3 ^{c}	5.3 ^{a}	15.8 ^{b}	17.6 ^{b}	7.5 ^{a}
	B	8.8 ^{a}	4.1 ^{ab}	2.4 ^{ab}	10.7 ^{a}	11.4 ^{bc}	5.4 ^{a}	8.8 ^{a}	7.6 ^{a}	4.8 ^{a}
	C	7.7 ^{a}	3.6 ^{ab}	1.6 ^{a}	9.6 ^{a}	4.6 ^{ab}	2.8 ^{a}	6.9 ^{a}	5.6 ^{a}	4.7 ^{a}
	D	7.7 ^{a}	2.3 ^{ab}	1.6 ^{a}	6.1 ^{a}	4.8 ^{ab}	2.7 ^{a}	6.7 ^{a}	6.1 ^{a}	4.6 ^{a}
	CC	8.8 ^{a}	1.5 ^{a}	2.8 ^{ab}	7.8 ^{a}	2.7 ^{a}	4.2 ^{a}	6.5 ^{a}	5.8 ^{a}	7.2 ^{a}

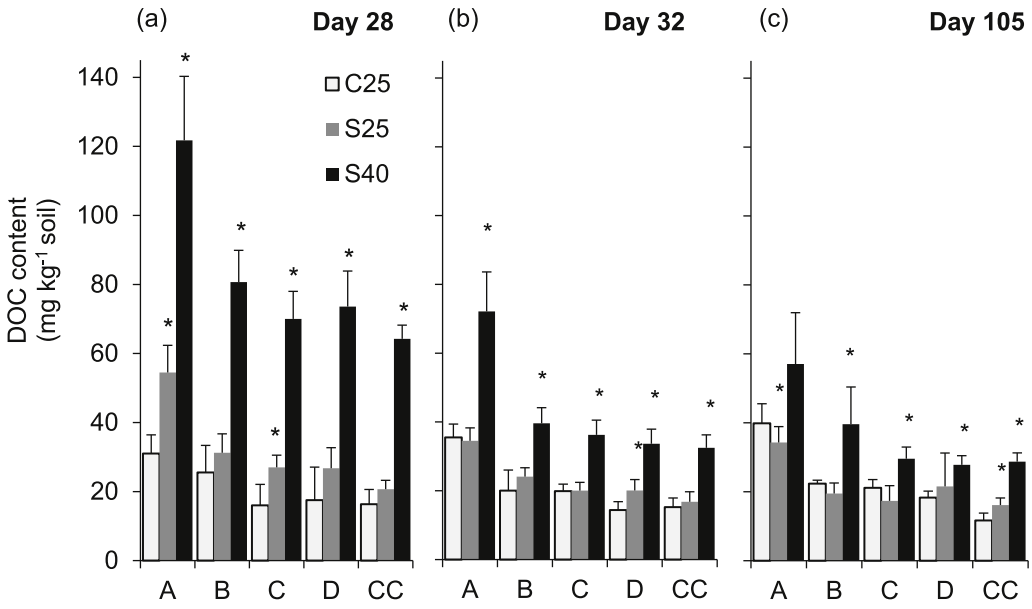

Fig. 3. DOC content in mg C kg^{-1} soil for the different positions (A, B, C, D and CC) under control (C25), drought stress (S25), and combined drought and heat stress (S40) conditions on days 28 (a), 32 (b) and 105 (c). Data are means +standard deviation (n = 5), and "*" indicates mean values that are significantly different from the control.

cumulative C mineralization presented similar trends under different treatments, while the position effect was buffered (Table 3).

The RS and RL indices based on C mineralization were systematically positive, did not show any significant stress treatment effect ($P >$ 0.05) and were mainly driven by the position factor or interaction of both position and treatment (Table 2). Soils from both stress treatments exhibited the highest RS index at position C. In S25, soils from positions A and B presented the highest RL indices. The lowest RL index was found for the C and CC positions while no differences in RL, according to position, appeared under S40 (Table 2).

After 28 days of drought, differences in DOC contents were mainly due to the treatment and, to a lesser extent, to the position: 68% and 8% of explained variance, respectively (data not shown). In the short term after rewetting, the position explained as much as the treatment and at the end of the experiment, the position being the main driver of DOC content (56% of explained variance) (data not shown). The DOC values after 28 days of drought at 40 °C were 3- to 4-fold greater ($P < 0.05$) than the control values (Fig. 3a). Drought stress increased soil DOC significantly at positions A and C at 25 °C and at all positions at 40 °C. Rewetting induced a significant decrease in the DOC content of S25 (Fig. 3b), compared to day 28 (Fig. 3a), reaching values similar to those of C25. This decrease in DOC was more important for soils maintained at 40 °C, but the DOC contents were still higher than those of soils incubated at 25 °C. At the end of the RL period, soils exposed to the S40 treatment still exhibited the highest DOC contents, ranging from 28 mg C kg^{-1} soil in position D to 57 mg C kg^{-1} soil at the tree row (Fig. 3c).

The position was the factor explaining most of the variance of mineral N concentration for each sampling date (between 47 and 69%), the treatment factor explaining between 19 and 46% of the total variance (data not shown). Relative to the control, on day 28, drought stress at 25 °C induced a N immobilization amounting to 14.7 (maximum) to 3.4 (minimum) mg N kg^{-1} soil at positions A and D, respectively (Fig. 4a). The addition of a heat stress (S40 treatment) slightly reduced the amount of N immobilized, which amounted to 11 (maximum) to 0.8 (minimum) mg N kg^{-1} soil at positions A and D, respectively. The rewetting induced a flush of N mineralization that was particularly high at 40 °C (Fig. 4b). At the end of the RL period, soils exposed to the S40 treatment still exhibited the highest mineral N concentration (Fig. 4c).

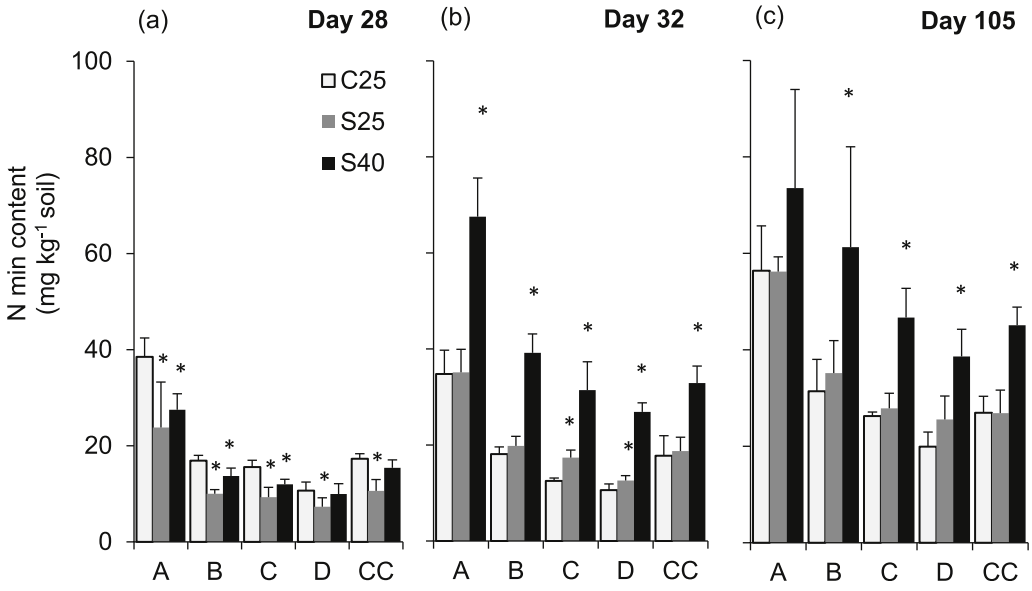

Fig. 4. Mineral N content in mg N kg^{-1} soil for the different positions (A, B, C, D and CC) under control (C25), drought stress (S25), and combined drought and heat stress (S40) conditions on days 28 (a), 32 (b) and 105 (c). Data are means +standard deviation (n = 5), and "*" indicates mean values that are significantly different from the control.

Table 5
Copy numbers of 16S and 18S rRNA genes (copy numbers g^{-1} soil) on day 28, after one month of stress conditions. The means for the different spatial positions (A, B, C, D, and CC) under control (C25), drought stress (S25), and combined drought and heat stress (S40) are presented. Sum of squares in percentages (%) of the two-ways ANOVA for position, treatment and interaction of both (P × T) are presented at the top of the table, with levels of statistical significance: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Data represent mean values (n = 5). Within a column, different lowercase letters indicate significantly different values between positions for each treatment ($P < 0.05$). Different capital letters indicate significant differences between treatments for each variable ($P < 0.05$). The '*' indicates a 16S or 18S abundance value that is significantly different from the control.

	Day 28					
Factor	Copy numbers of 16S			Copy numbers of 18S		
Position	47 ***			34 ***		
Treatment	43 ***			36 ***		
P × T	1			8 **		
Position	C25 ^{A}	S25 ^{B}	S40 ^{C}	C25 ^{A}	S25 ^{A}	S40 ^{B}
A	$1{,}10 \times 10^{10}$ ^{c}	$8{,}88 \times 10^{09}$ ^{c} (- 19%) *	$6{,}46 \times 10^{09}$ ^{b} (- 41%) *	$9{,}68 \times 10^{07}$ ^{b}	$7{,}06 \times 10^{07}$ ^{c} (- 27%) *	$3{,}53 \times 10^{07}$ ^{b} (- 64%) *
B	$7{,}89 \times 10^{09}$ ^{b}	$6{,}04 \times 10^{09}$ ^{b} (- 23%) *	$3{,}60 \times 10^{09}$ ^{a} (- 54%) *	$6{,}62 \times 10^{07}$ ^{b}	$5{,}98 \times 10^{07}$ ^{bc} (- 10%)	$2{,}40 \times 10^{07}$ ^{ab} (- 64%) *
C	$7{,}05 \times 10^{09}$ ^{ab}	$4{,}99 \times 10^{09}$ ^{ab} (- 29%) *	$3{,}25 \times 10^{09}$ ^{a} (- 54%) *	$4{,}70 \times 10^{07}$ ^{a}	$4{,}12 \times 10^{07}$ ^{ab} (- 12%)	$1{,}98 \times 10^{07}$ ^{a} (- 58%) *
D	$6{,}28 \times 10^{09}$ ^{ab}	$4{,}68 \times 10^{09}$ ^{ab} (- 25%) *	$2{,}83 \times 10^{09}$ ^{a} (- 55%) *	$4{,}10 \times 10^{07}$ ^{a}	$3{,}58 \times 10^{07}$ ^{ab} (- 13%)	$2{,}23 \times 10^{07}$ ^{a} (- 45%) *
CC	$5{,}70 \times 10^{09}$ ^{a}	$4{,}31 \times 10^{09}$ ^{a} (- 24%) *	$2{,}94 \times 10^{09}$ ^{a} (- 48%) *	$3{,}85 \times 10^{07}$ ^{a}	$3{,}38 \times 10^{07}$ ^{a} (- 12%)	$1{,}90 \times 10^{07}$ ^{a} (- 51%) *

3.3. Soil microbial biomass C, N and P contents, resistance and resilience

The MBC was more affected by the position factor (between 50 and 54%) than the treatment factor (between 22 and 28%) over the experiment while the MBN was more driven by the treatment factor (between 44 and 59%) (Table 4). Total variance of MBP was more driven by the treatment at day 28 and 32 and more by both the position and treatment at the end of the experiment. At day 28 under S40, compared to the control (C25), MBC was decreased by 24 (CC) to 48% (A), while it was decreased by 9 (CC) to 20% (A) under S25. The MBN was also more affected under S40 (between 83 and 91% of decrease) than S25 (between 60 and 72% of decrease), while MBP did not show significant difference between S25 and S40. After rewetting and at the end of the incubation experiment the microbial biomass C, N and P contents were still lower under S40 treatment than under S25 or C25.

The RS indices of MBC and MBN were mainly driven by the treatment factor while the RS indices of MBP was not significantly affected by any of the two factors. The RS indices for MBC, MBN and MBP at 25 °C did not show any significant position effect (Table 6). The heat stress strongly decreased the RS of soil MBC and MBN. Soils from S40 exhibited the highest RS of MBC in the D and CC positions (0.64 and 0.73 respectively) and highest RS of MBN in the B and CC positions (0.09 for both). The RS of MBC was significantly higher than the RS of MBN and MBP ($P < 0.05$ data not shown). The RL indices differed according to the position factor for MBC while it differed according to the treatment for MBN and MBP (i.e. RL was higher under S25 than S40 treatment). The RL indices for MBC were lowest on D and CC positions (Table 6).

3.4. Elemental ratios of microbial biomass (C, N and P) and homeostasis

The position had a minor effect on the microbial biomass elemental ratios throughout the experiment, explaining between 1 and 8% of the variance, while the stress treatments explained between 30 and 56% of the variance of the MBC:MBN ratio (Table S1). When P was accounted for in the ratios, the treatment explained less than 12% of the variance. After the first drought stress period and before rewetting (day 28), the slope of MBC:MBN was almost twice higher in S25 and S40 than in C25 (Fig. 6a, Table S2), while the mean MBC:MBN values increased from 6 to 62 (Table S1). This was mainly due to the strong decrease in MBN compared to MBC in response to drought and heat stress (Fig. 6a). In response to drought at 25 °C, the mean values of MBC:MBP also showed a significant increase, due to higher decrease of MBP than MBC (Fig. 6b), approximately 2-fold or more for A, B, C or D positions and more than 7-fold for the CC position (127) (Table S1). The value of MBN:MBP ratio and its slope significantly decreased from the control to the S40 treatment due to higher decrease in MBN compared to MBP, while it increased under the S25 treatment (Fig. 6c, Table S2). Rewetting at 25 °C induced a recovery, as the S25 slopes were similar to the control slopes (C25), whereas none of the S40 slopes were significant (Fig. 6d, e, f, Table S2). The same pattern occurred at the end of the incubation, i.e., the S25 and C25 soils exhibited similar microbial biomass elemental ratios and associated slopes, which differed from those of the S40 soils, because of lower RL of MBN and MBP (Fig. 6g, h, i, Tables S1 and S2).

Relationships between the microbial biomass elemental ratios (log molar basis) and the soil elemental ratios (log molar basis) showed slopes that were not significantly different from 0 in any treatment, except for one case ($P = 0.04$), indicating the global homeostatic behavior of soil microorganisms regardless of the type of stress applied (Fig. S3).

3.5. Fungal and bacterial abundances based on 16S rRNA and 18S rRNA genes

The 16S rRNA and 18S rRNA genes were successfully amplified with PCR efficiencies of 98% and 92%, respectively, and a standard curve R^2 value of 0.999 for both. Bacterial and fungal abundances always reached the highest values at the tree row (A position), regardless of the treatment (Table 5). The total variance of copy numbers of 16S and 18S were as much explained by the position (47 and 34%) than by the treatment factor (43 and 36%) (Table 5). The ordination plots of RDA clearly distinguished two groups according to the stress treatment (S25 or S40) (Fig. 7). The RSs of MBC, MBN and MBP were positively linked to MBC, MBN and MBP while they were negatively linked with DOC contents and with the decrease in copy number of both 16S and 18S (Fig. 7). Distinction between positions was different according to the treatment: under S25, B position was intermediate to A and all other positions while under S40, A position differed from all other positions. After 28 days of drought stress at 25 °C, the 18S abundance of the stress

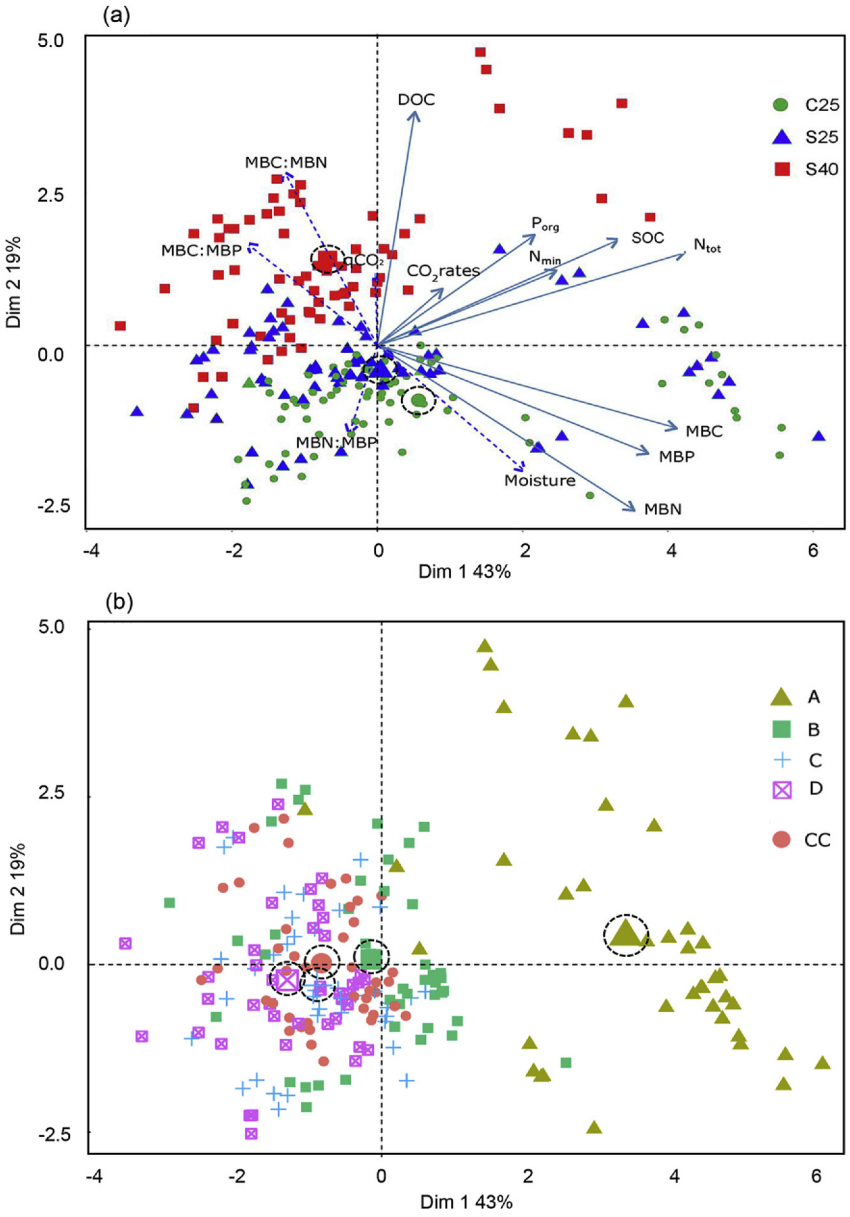

Fig. 5. Principal component analysis (PCA) performed for MBC, MBN, MBP, organic C mineralization (CO_2 rates), DOC content, SOC content, organic P (P_{org}) content, mineral (N_{min}) and total N (N_{tot}) content as explanatory variables (solid arrows) with the microbial biomass ratios (MBC:MBN, MBC:MBP, MBN:MBP), metabolic quotient (qCO_2) and soil moisture as supplementary quantitative variables (dotted arrows). Symbols indicate treatment (a) and spatial position (b), and centroids are circled by a dotted line.

treatment was significantly lower than that of the control only at the tree row position (−27%) (Table 5). The 16S and 18S abundances were significantly lower under the S40 treatment than under the S25 treatment ($P < 0.05$). The decrease in the abundance of 18S was greater than that of 16S at the tree row (A position) under the S25 treatment and at the tree row and B position under the S40 treatment ($P < 0.05$). The 16S:18S ratio was not influenced by the position and was altered only by the S40 treatment (data not shown).

3.6. Metabolic quotient

The treatment factor was the main driver of variation of the metabolic quotient (qCO_2) (Table 7) which thus were strongly affected by drought stress at both 25 °C and 40 °C. On day 28, the stress-exposed values were significantly lower than the control values for all positions, and qCO_2 was more affected by the S25 than by the S40 treatment. Three days after rewetting (day 32), the qCO_2 values of both stress treatments were much higher than that of the control. The qCO_2 values increased 2- to 3-fold in response to drought at 25 °C, and they increased 7- to 9-fold at 40 °C. At the end of the incubation, the qCO_2 in S25 decreased to values close to those of the control, while in S40, the qCO_2 values remained 1.2- to 1.7-fold higher than those of C25. After 28 days of drought, the highest qCO_2 values were found in C position for both S25 and S40 treatments. Three days after rewetting (day 32), the lowest value was found in CC position while the highest value was found in B position (Table 7).

4. Discussion

4.1. Spatial heterogeneity of soil properties, as induced by agroforestry management

The introduction of tree rows into arable land can generate spatial heterogeneity in nutrient availability and microbial activity. In the

Table 6
Resistance (RS) and resilience (RL) indices based on microbial biomass C (MBC), N (MBN) and P (MBP) contents at different spatial positions (A, B, C, D and CC) for the S25 and S40 treatments. Resistance indices were calculated on day 28, and resilience indices were calculated on day 105. Sum of squares in percentages (%) of the two-ways ANOVA for position, treatment and interaction of both (P × T) are presented at the top of the table, with levels of statistical significance: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Data represent mean values (n = 5). Different letters in a column indicate significant differences in RS or RL between positions for each treatment ($P < 0.05$).

	Day 28						Day 105					
Factor	RS MBC		RS MBN		RS MBP		RL MBC		RL MBN		RL MBP	
Position	19 ***		4		6		22 *		1		13	
Treatment	47 ***		70 ***		3		< 1		70 ***		12 *	
P × T	15 ***		8 **		18		18		4		10	
Position	S25 ^{A}	S40 ^{B}	S25 ^{A}	S40 ^{B}	S25 ^{A}	S40 ^{B}	S25 ^{A}	S40 ^{A}	S25 ^{A}	S40 ^{B}	S25 ^{A}	S40 ^{B}
A	0.71 ^{a}	0.36 ^{a}	0.23 ^{a}	0.05 ^{a}	0.23 ^{a}	0.18 ^{a}	0.6 ^{a}	0.3 ^{bc}	0.7 ^{a}	0.3 ^{a}	0.7 ^{a}	0.3 ^{a}
B	0.83 ^{a}	0.38 ^{a}	0.26 ^{a}	0.09 ^{b}	0.36 ^{a}	0.17 ^{a}	0.1 ^{a}	0.2 ^{bc}	0.7 ^{a}	0.2 ^{a}	0.7 ^{a}	0.3 ^{a}
C	0.76 ^{a}	0.51 ^{b}	0.24 ^{a}	0.05 ^{a}	0.32 ^{a}	0.12 ^{a}	0.3 ^{a}	0.4 ^{c}	0.8 ^{a}	0.2 ^{a}	0.3 ^{a}	0.5 ^{a}
D	0.77 ^{a}	0.64 ^{c}	0.20 ^{a}	0.04 ^{a}	0.18 ^{a}	0.12 ^{a}	-0.1 ^{a}	0.1 ^{ab}	0.7 ^{a}	0.3 ^{a}	0.7 ^{a}	0.4 ^{a}
CC	0.81 ^{a}	0.73 ^{c}	0.16 ^{a}	0.09 ^{b}	0.09 ^{a}	0.20 ^{a}	0.2 ^{a}	-0.1 ^{a}	0.8 ^{a}	0.2 ^{a}	0.8 ^{a}	0.6 ^{a}

present agroforestry system, 21 years after tree rows' plantation, such heterogeneity was detectable at the SOM level, soil respiration, inorganic N and microbial biomass C, N, and P contents. Higher concentrations of SOC and total N were measured in the tree row than in the middle of the interrow (D position), while the soil texture characteristics remained unchanged. A previous study performed at the same site (Cardinael et al., 2015) also found higher SOC concentrations at the tree row (21.6 mg C g^{-1} soil) than in the middle of the interrow (9.8 mg C g^{-1} soil) or in the CC (9.3 mg C g^{-1} soil). We measured similar SOC concentrations at the tree rows (20.5 mg C g^{-1} soil), but the values in the middle of the interrow (13.9 mg C g^{-1} soil) and in the CC (15.2 mg C g^{-1} soil) were higher than the values reported by Cardinael et al. (2015). This can be explained partly by differences in sampling design. Indeed, the results of Cardinael et al. (2015) were based on a 625 m^2 subplot with 9 walnut trees spread across 2 tree rows in an agroforestry system. Our sampling design was based on 5 transects spread over an entire agroforestry plot (4.6 ha), thus exhibiting greater soil spatial heterogeneity. The similar observation for the CC indicates that the SOC concentrations at the tree rows were more homogeneous than those in the cropped area. The higher levels of SOM and total N at the tree rows could be related to i) the absence of tillage, which could favor both the accumulation and protection of organic C and N in the surface soil (Cookson et al., 2008; Dimassi et al., 2013), ii) the presence of a spontaneous and perennial herbaceous cover under the tree row. Such covers provide labile C through root exudation (Pausch and Kuzyakov, 2018) as well as high litter inputs compared to the exported biomass in the interrow and iii) the biomass of falling leaves and herbaceous cover, which could provide relatively slowly decomposing material for microorganisms, thus resulting in an accumulation of organic C and total N (Mungai and Motavalli, 2006).

Compared to the global analysis of Xu et al. (2013), which summarized the soil microbial biomass of major biomes on Earth, the high microbial biomass C, N, and P measured at the tree row in our study (mean molar values (in mmol CNP kg^{-1} soil) of control soils, MBC: 30.4, MBN: 3.9, MBP: 0.5) were close to those reported for shrubs (molar values (in mmol C, N or P kg^{-1} soil), MBC: 28.5, MBN: 7.2, MBP: 0.6). The other positions of the spatial gradient and the CC in our study showed similar microbial biomass C, N, and P contents, with values slightly lower than those reported by Xu et al. (2013) for cropland. However, the molar C:N:P ratio of the microbial biomass in the tree row control soils (59:8:1) or in the cropped interrow and CC soils (mean ratio of 66:8:1) in our study were higher than those presented by Xu et al. (2013) for shrub (43:6:1) and cropland (38:5:1), thus indicating that the higher MBC:MBP and MBN:MBP ratios could be related to the low soil P content of our study site. Nevertheless, the average molar C:N:P ratio of the microbial biomass in the control soils across all positions was 65:8:1, which is consistent with the reference value of 60:7:1 proposed by Cleveland and Liptzin (2007), although these authors did not consider cropped sites in their study.

4.2. Resistance and resilience of soil microbial communities after drying-wetting cycles

Both stresses led to similar RS when considering C mineralization rates after 28 days of stress, while microbial biomass C, N and P were more impacted by the combination of heat and drought stress than by drought stress alone. When microorganisms experience stress, they can allocate more C and nutrients to survival pathways than to growth and resource acquisition pathways (Schimel et al., 2007; Tiemann and Billings, 2011). A survival regime implies the synthesis of specific molecules such as polyols or amino-acids that are energetically expensive and, consequently, lower CO_2 emissions and qCO_2. In our work, combined drought and heat stress (S40) probably generated a high mortality, thus explaining the higher level of DOC, which was possibly due to the lysis of microbial cells (West et al., 1989), as confirmed by a decrease in MBC after 28 days of stress. We found that the fungal community was less affected by drought conditions than the bacterial community, and therefore was more resistant, which is in agreement with de Vries et al. (2012) and Kaisermann et al. (2013). Under combined drought and heat stress, the decrease in fungal abundance was significantly greater than the decrease in bacterial abundance at and near the tree row, which could help explain the lower RS of MBC that we observed at and near the tree row.

After rewetting, flushes of C and N mineralization (Fig. 2b and c; 4b) were observed in both stress treatments, and heat strongly amplified the phenomenon, suggesting that C and N were not limiting biomass growth. This "Birch effect" had already been reported in many studies investigating the effects of drying-wetting cycles (Birch, 1958; Fierer and Schimel, 2002; Xiang et al., 2008). Most studies reported that frequent drying-wetting cycles decreased the CO_2 pulses after rewetting due to the limitation of the accessible SOM pool (Borken and Matzner, 2009; Butterly et al., 2009); however, this was not the case for drought stress alone. The effects of drying-wetting cycles on MBC vary in the literature (Butterly et al., 2009; Sawada et al., 2017). Two different mechanisms with opposite effects can occur: MBC can be reduced due to stress-sensitive microorganisms (Van Gestel et al., 1993) or increased due to substrate release via the breakdown of aggregates and release of previously protected SOC (Denef et al., 2001). Although these two phenomena are difficult to distinguish, our results showed, after rewetting, the DOC of S25 was equal to that of the control, while the DOC

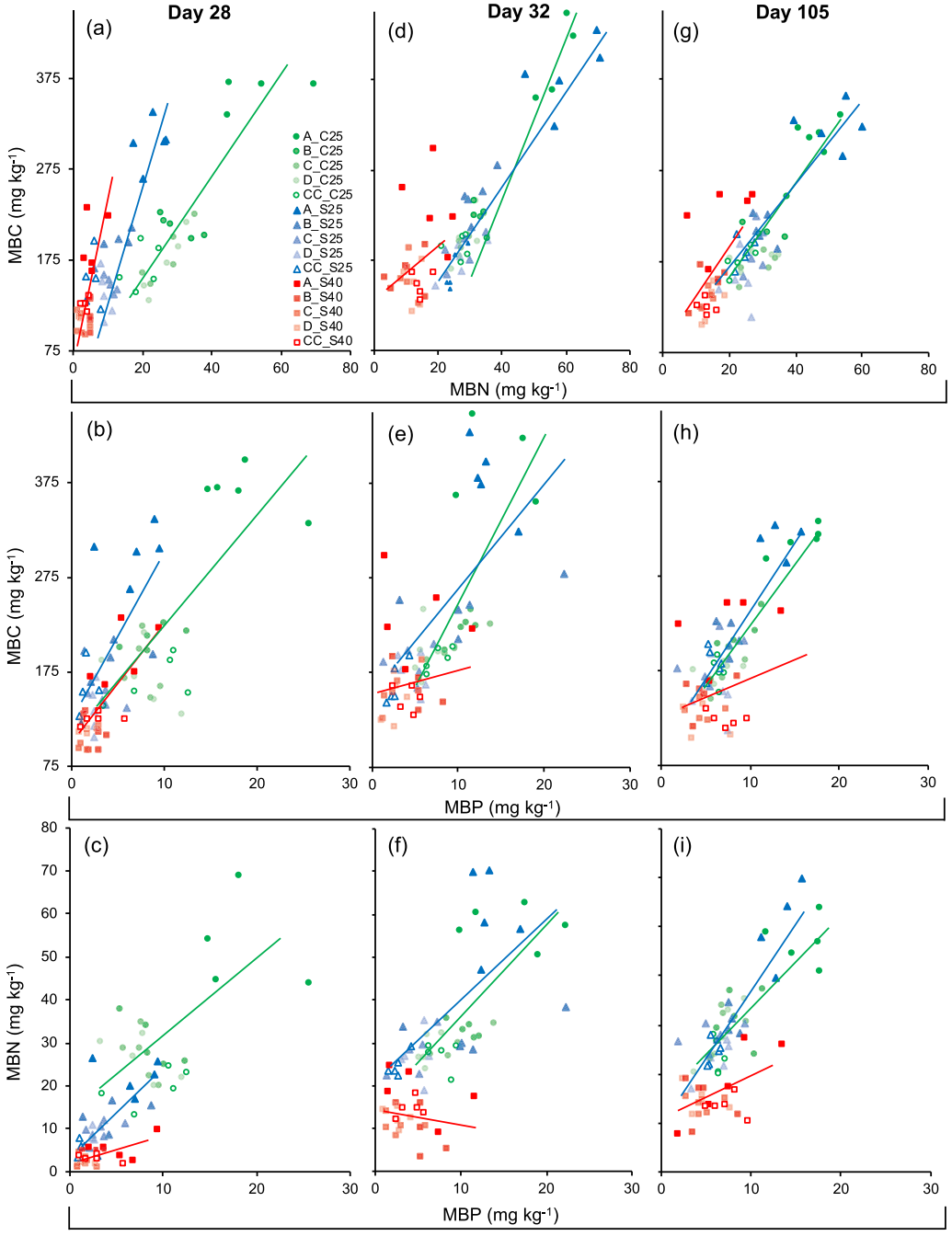

Fig. 6. Scatter plot of relationship between microbial biomass C, N or P contents for C25 (green circles, n = 25), S25 (blue triangles, n = 25) and S40 (red squares, n = 25). Microbial biomass was expressed in mg C, N or P kg^{-1} soil. Graphs a, b and c represent relationship on day 28, graphs d, e, and f on day 32, and graphs g, h, and i on day 105. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

in S40 was higher than that of the control; this again suggests that the SOC concentration did not limit the growth of soil microorganisms. After the second rewetting, soils previously exposed to drought and heat stress presented higher CO_2 pulses than those exposed only to drought stress, suggesting a lasting effect of temperature on microbial functioning.

The concomitant high qCO_2 and low MBC recovery at the tree row under combined drought and heat stress indicate that microorganisms invested more in energetic maintenance than in biomass growth. This response pattern was opposite in the CC, which demonstrated relatively high MBC recovery rates (data not shown) and the lowest qCO_2. These observations support the occurrence of microbial adaptation, and are inconsistent with our initial hypothesis, which suggested that soil microbial communities from the tree rows would be more resistant to stress than those from the middle of the interrow or the CC. In field conditions, the presence of trees and permanent herbaceous cover along the tree rows could buffer variations in moisture and temperature at the soil surface. Milder microclimatic conditions with than without trees have already been reported in silvopasture and agroforestry systems (Karki and Goodman, 2015; Ong et al., 2000). For instance, at our experimental site, the day-time topsoil temperatures recorded in June 2017 were 3 °C lower in the agroforestry system than in the CC system (Marie Gosme, personal communication), and soil moisture always decreased by 1–3% with increasing distance from the tree row. Because

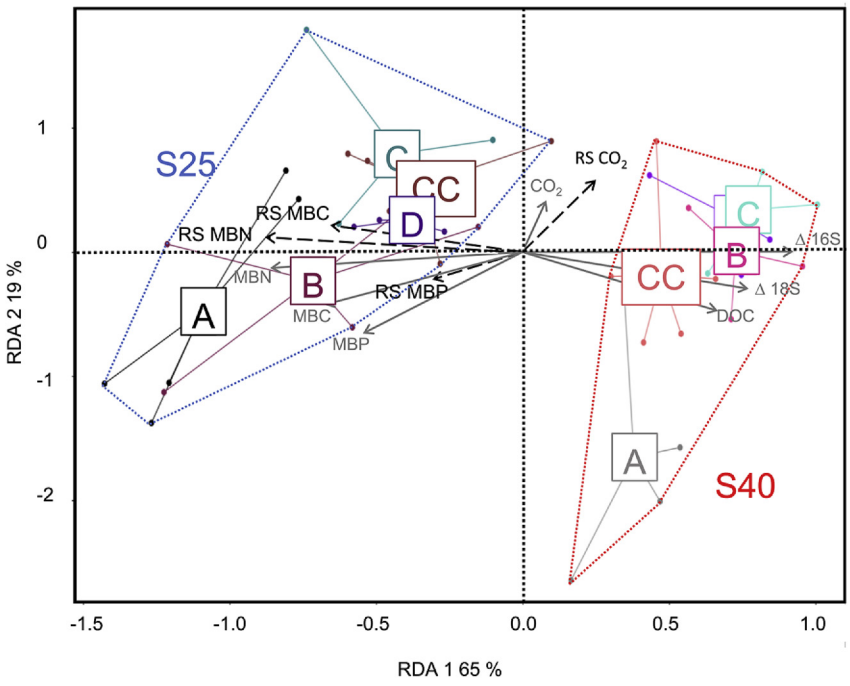

Fig. 7. Redundancy analysis (RDA) based on RS of microbial biomass C (RS MBC), N (RS MBN) and P (RS MBP) and CO_2 mineralization (RS CO_2) constrained by DOC content, decrease of 16S (Δ16S) and 18S (Δ18S) copy numbers, microbial biomass C (MBC), N (MBN), P (MBP) and microbial respiration (CO_2). The RDA was performed on center-reduced matrix. Different spatial positions (A, B, C, D and CC) are represented for S25 treatment, surrounded by a blue dotted line and for S40 treatment, surrounded by a red dotted line. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

soil moisture has been shown to induce a legacy effect on soil microbial community structure and processes (Banerjee et al., 2016), one may expect the soil microorganisms sampled at the tree row to be less adapted to very dry and hot conditions than those sampled in the middle of the interrow or in the CC, leading to a lower RS to these combined stress conditions.

Regarding the MBC, the soil microbial communities in the middle of the interrow and the CC exhibited a highest RS and lowest RL under combined drought and heat. While the position effect on RS is likely related to microbial adaptation to climatic conditions, the opposite response of RL is probably related to differences in resource availability (Orwin et al., 2006). The high release of available resources such as DOC or mineral N due to cell death after combined drought and heat stress, especially at and near the tree row, could be rapidly used by microorganisms to growth.

Ingrisch and Bahn (2018) divided metrics of the resilience in 3 categories according to the component of recovery they address: normalized system state, baseline-normalized system state and impact-normalized system state. We designed our experiment to evaluate the RL at the end of the experiment by the third approach. The RL index can thus be negative if the absolute value of difference between the soil under stress treatment and control treatment is higher at the end of the experiment than during the resistance period. This explains some of the low RL microbial biomass indices observed in the present study, with similar values in the control soils and soils under the stress treatments, as well as the high variability among replicates.

4.3. Impact of stress on microbial biomass C, N, and P contents and ratios

To our knowledge, this is the first time that a study has analyzed the response of elemental ratios of soil microbial biomass to drought and drought combined with heat stress. Our results exhibited some changes in these elemental ratios in response to both treatments. After one month, the slopes of MBC:MBN increased almost 2-fold under both stresses, but the S25 soils maintained a high R^2 of 0.7, indicating the S25 microbial communities were better constrained than the S40 communities (R^2 = 0.27). The greater decrease in MBN than in MBC (slope increase) could indicate a selection of microorganisms with high

Table 7
Metabolic quotients (qCO_2) in μg C-CO_2 mg^{-1} MBC h^{-1} at different spatial positions (A, B, C, D and CC) under control (C25), drought stress (S25) and combined drought and heat stress (S40) conditions on the 3 sampling dates. Sum of squares in percentages (%) of the two-ways ANOVA for position, treatment and interaction of both (P × T) are presented at the top of the table, with levels of statistical significance: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Data represent mean values (n = 5). Within a column, different lowercase letters indicate significantly different values between positions for each treatment ($P < 0.05$). Different capital letters indicate significant differences between treatments for each variable ($P < 0.05$).

	Day 28			Day 32			Day 105		
Factor									
Position	3			1			9		
Treatment	46 ***			74 ***			15 **		
P × T	13 *			4			14		
Position	C25 ^{A}	S25 ^{C}	S40 ^{B}	C25 ^{A}	S25 ^{B}	S40 ^{C}	C25 ^{A}	S25 ^{AB}	S40 ^{B}
A	1.1 ^{a}	0.2 ^{ab}	0.2 ^{a}	1.3 ^{a}	2.9 ^{a}	12.2 ^{ab}	0.9 ^{ab}	1.0 ^{a}	1.6 ^{a}
B	1.2 ^{a}	0.1 ^{a}	0.5 ^{b}	1.7 ^{a}	4.8 ^{a}	13.8 ^{b}	1.1 ^{b}	1.1 ^{a}	1.6 ^{a}
C	1.0 ^{a}	0.4 ^{b}	0.6 ^{b}	1.3 ^{a}	3.4 ^{a}	10.9 ^{ab}	1.0 ^{b}	1.1 ^{a}	1.2 ^{a}
D	1.1 ^{a}	0.2 ^{a}	0.5 ^{ab}	1.5 ^{a}	4.1 ^{a}	10.6 ^{ab}	1.0 ^{ab}	1.0 ^{a}	1.2 ^{a}
CC	1.1 ^{a}	0.2 ^{a}	0.3 ^{ab}	1.3 ^{a}	4.1 ^{a}	9.3 ^{a}	0.8 ^{a}	1.1 ^{a}	1.0 ^{a}

MBC:MBN ratios and low N demand. The low immobilization as well as the low NUE (data not shown), which was calculated according to Mooshammer et al. (2014), demonstrated that there was no N limitation. S40 had the lowest slope of MBN versus MBP, which suggests that microbial N was more affected than microbial P in these conditions. However, changes in the MBC:MBP or MBN:MBP ratios of microorganisms are usually caused by P content changes rather than C or N content changes following the growth rate hypothesis (Sterner and Elser, 2002). Although the soil available P (Olsen P) concentration was relatively low (between 5.2 and 8.3 mg kg^{-1} soil), microorganisms under stress modified more their N than their P content, which suggests that the low soil P availability was not the main driver of microbial biomass functioning.

Under the S25 treatment, rewetting favored the growth of microorganisms, which presented flexible elemental ratios, i.e., ratios that returned to values close to those of the control soils only 3 days after rewetting (Fig. 6d, e, f). The combination of drought and heat stress induced microbial growth under stressful conditions. The considerably high variability in elemental ratios of microbial biomass could suggest that microorganisms were no longer constrained at this moment. This effect lasted until the end of the incubation experiment. Differences in soil microorganism functioning were found when comparing the two different stress treatments after rewetting, the growth rate hypothesis being thus confirmed only under the S25 treatment. This hypothesis stipulates that fast growth is negatively correlated to MBC:MBP and MBN:MBP (Sterner and Elser, 2002). We associated growth rate to MBC growth between day 28 and day 32 and MBC:MBP or MBN:MBP to the mean of these ratios at day 28 and 32. The negative correlation was verified by the S25 treatment only. It was not the case for the S40 treatment, probably due to the high mortality, low growth rate and selection of specific microbial communities, which may not follow the growth rate hypothesis at such a short time scale. Interestingly, although the studied stresses strongly affected microbial biomass C:N, C:P and N:P ratios, the overall microbial community remained homeostatic.

Microbial biomass C:N, C:P and N:P ratios could be used as a proxy of stressful conditions, although this should be assessed using a larger range of soils and stress conditions. At the end of our experiment, all slopes corresponding to the MBC:MBN, MBC:MBP and MBN:MBP ratios of the S25 microorganisms were similar to those of the control microorganisms, suggesting a high flexibility of these communities against drought. In contrast, the impact of combined drought and heat stress, even after one month under optimal conditions, was still observable. The slopes of MBN:MBP and MBC:MBP were still lower for soils that had been exposed to this combined stress than for those of the controls soils. In the present work, the soil microbial communities thus exhibited much less stoichiometric flexibility under combined drought and heat stress than under drought stress only.

5. Conclusion

Our study showed that an agroforestry system can induce spatial heterogeneity in the soil microbial response to drought stress and combined drought and heat stress. Compared to soils from other positions, soils sampled at and close to the tree row always exhibited the highest microbial biomass and mineralization activity during the stress period, although this trend, as we demonstrated, was not associated with a higher RS. When exposed to drought only, soil microorganisms from all positions, including the conventional crop, presented similar resistance of microbial biomass. However, when heat and drought stress were combined, the microorganisms at and near the tree row that are presumably accustomed to less stressful conditions *in situ* exhibited lower resistance in *ex situ* conditions. Microorganisms from the middle of the inter-row and the conventional crop showed a stronger adaptation to dry and hot conditions and were less impacted during disturbance periods in the middle of the interrow and in the conventional crop than at the tree row. Another major finding of the present study is that soil microbial biomass and functions were modified beyond the tree row, especially in the adjacent first meter of the interrow. For the first time, we demonstrated that soil microorganisms can considerably change their stoichiometry in response to different stress treatments. They exhibited high stoichiometric flexibility under drought stress only, while irreversible stoichiometric changes were observed under combined drought and heat stress. The potential to use such changes in stoichiometry at the community level as indicators of climatic stress should be further investigated.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2018.11.011.

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